DRAFT

Working Group 1b. Liver Injury, Repair, Fibrosis & Inflammation

Introduction & Background

Tissue injury and inflammation, repair, and fibrosis are fundamental components of acute and chronic liver diseases regardless of cause. Hepatocellular injury and death, with the accompanying inflammatory response, provoke symptoms of fatigue and weakness, and account for elevations of serum aminotransferases that are characteristic of liver disease. More importantly, hepatic injury and inflammation also lead to loss of liver function. The ensuing responses of cell repair, regeneration and fibrosis ultimately determine whether patients recover from liver disease or develop progressive scarring of the liver, which can result in cirrhosis and portal hypertension. Clarifying the cellular and molecular mechanisms that underlie these processes is critical for designing and developing effective treatments for patients with liver disease. In some instances, therapies can be targeted against the specific agents that cause liver disease, such as the hepatitis B and C viruses. In most liver diseases, however, therapy against intermediate processes mediating liver injury would greatly benefit patients. Such therapies would include means to decrease cell injury and inflammation, promote regeneration and repair, and ameliorate fibrosis. Because the mechanisms of injury and repair are similar among a variety of liver diseases, therapies directed against these pathways are likely to be helpful for a wide range of conditions. Drugs that prevent liver cell death, inhibit liver fibrosis and promote repair will emerge only from a detailed understanding of these processes, including the complex, interwoven pathways involved. New therapies that result from careful research into the mechanisms of liver cell death, hepatic inflammation, fibrosis and repair could have an immense salutary impact on human health and disease, both by saving lives and by substantially reducing health care expenditures.

Recent Research Advances

The last decade has witnessed tremendous progress toward uncovering the fundamental mechanisms that contribute to liver injury, repair, inflammation and fibrosis. Despite this progress, new insights have not been sufficiently translated into innovative therapies. Unlike breakthroughs in other major biomedical fields, such as atherosclerosis and cancer, advances in liver cell biology have not yet had a meaningful impact on the natural history or prevalence of liver disease. Progress to date must be extended to uncover new tools for basic discoveries and opportunities for translational advances. For example, solid evidence has accumulated demonstrating that apoptotic cell injury is generic to many liver diseases, sinusoidal cells are critical for hepatocyte survival, hepatic stellate cells/myofibroblasts are a key source of collagen deposition in liver fibrosis, and hepatic fibrosis has a reversible component. An understanding of progenitor cell biology and the role of these cells in tissue repair has also emerged. Critical intracellular signaling cascades culminating in apoptotic cell death, inflammatory cell recruitment and collagen

production have been elucidated. The extracellular matrix has been found to play an important role in cell signaling and the repair process. Disease-specific and cell type-specific responses have become apparent, which may allow some therapeutic targeting to these pathophysiologic events. However, substantive gaps in our knowledge persist and more progress should be pursued through additional and expanded research initiatives. Although several ligand/receptor-initiated intracellular signaling cascades and gene expression profiles have been characterized, we know little about which pathways are dominant in specific liver cell populations *in vivo*, how these pathways interact functionally and what impact they have on expression and persistence of disease. Even more striking is our lack of knowledge about the innate and adaptive immune response to liver injury from viruses or metabolic and autoimmune diseases.

Obvious questions persist in these and other areas, such as: How do the innate and adaptive immune systems responses react to and modulate apoptosis, liver repair, hepatic metabolism and fibrosis? Can deleterious aspects of metabolism, including its resultant generation of reactive nitrogen and oxygen species, be modified to minimize disease progression? How do apoptosis and oncotic necrosis influence fibrosis? What is the basis for the remarkable property of hepatic regeneration that distinguishes the liver from all other solid organs? What are the source(s) and fates of progenitor cells in liver injury? How can specific liver toxicities be predicted and prevented? What are the genetic determinants of liver disease, and how does the interplay between genes and environment influence the expression of liver disease?

There are also important deficiencies in the tools available to address these questions. For example, the field has been hampered by a lack of definitive and relevant disease models, which limits our ability to translate scientific information into therapeutic opportunities. Databases of genetic information focused on liver disease have yet to be developed, and tools to apply genetic information to the discovery of disease mechanisms are lacking. Finally, studies performed in cellular models require extension and implementation in whole organ and animal models.

Research Goals

The major research goals in liver injury, repair, inflammation and fibrosis are to understand the cellular mechanisms mediating these processes and to develop effective means for monitoring and treating the diseases caused by these processes.

Pathophysiology: Insights into liver injury will require information regarding the intra- and inter-cellular responses to toxic stimuli. Liver damage is frequently characterized by cell type-specific injury. For example, hepatocytes are the primary target of attack in viral hepatitis, cholangiocytes in primary biliary cirrhosis, and endothelial cells in sinusoidal obstruction syndrome (veno-occlusive disease). Major gaps remain in our knowledge of the fundamental responses of specific liver cell populations to injury and how cell injury leads to inflammation. Thus, important research goals are to identify cell type-specific cytotoxic signaling pathways in the liver and to determine how liver cells

produce and respond to inflammatory mediators (Matrix Cells A1 & B1). Ideally, these cell-specific pathways would be correlated with changes in genomic and proteomic patterns of expression, with care to include the analysis of post-translational modification of proteins by glycosylation, phosphorylation and oxidative or nitrosative processes. Not only do liver diseases provoke cell type-specific responses, but many disease processes affect more than one hepatic cell type, thus provoking complex intercellular responses. For example, activated resident macrophages known as Kupffer cells can generate reactive oxygen species and cytokines, which in turn injure adjacent parenchymal cells. Also, inflammatory stimuli can induce enzymes that generate nitric oxide, leading to nitrosative stress. Perturbations of intrahepatic blood flow commonly accompany clinical liver disease and are especially germane to the organ preservation injury that can occur in the setting of liver transplantation. To understand these intercellular responses and their consequences, future research might focus on the integrative mechanisms mediating oxidative, nitrosative, hypoxic, and ischemia-reperfusion injury. Certain cell types in the liver, such as endothelial and Kupffer cells, also play important roles in these processes and deserve careful assessment (Matrix Cell B2). Liver injury also provokes infiltration and adhesion of circulating leukocyte populations into the liver, which can modify and amplify the injury response. The impact of leukocyte sub-populations (neutrophils, cytotoxic T lymphocytes, natural killer cells, macrophages, etc) and their mediators on liver injury, fibrosis, and regeneration would benefit from further elucidation (Matrix Cell A2). New discoveries in this area will likely yield novel therapeutic targets and approaches.

S*mall molecules as therapeutic agents:* Small molecules have proven useful as therapeutic agents in a wide range of human afflictions. The pharmaceutical and biotechnology industries are often sources of such agents; however, opportunities to develop promising therapeutics for liver disease have not been uniformly pursued. To remedy this situation, high-throughput screens could be made available to investigators in academic medical centers in order to identify candidate small molecules capable of modifying cytotoxic and fibrotic pathways and of regulating the repair response in liver cells (Matrix Cell C2). Thus, a key research goal is for investigators to partner with industry whenever possible in developing high-throughput screens relevant to liver disease. Such efforts could provide cost-effective opportunities for pursuing selected compounds as therapies in preclinical studies.

Preclinical studies: Although reductionist, cell-based experimental systems are vital for elucidating signaling pathways that culminate in liver cell injury and fibrosis, the complex, integrated processes occurring in disease can only be fully evaluated in animal models. Furthermore, therapeutic endpoints can only be rigorously assessed *in vivo*. Therefore, an important research aim is to develop relevant and robust animal models that faithfully mimic the development and resolution of human hepatic injury and fibrosis (Matrix Cell C1). These models will accelerate the development of anti-apoptotic and hepatoprotective therapies that decrease liver injury and fibrosis, promote regeneration, and ameliorate other liver abnormalities (Matrix Cell A2). Acute and chronic animal models will also enable the *in vivo* testing of gene, cell-based and pharmacologically targeted therapies for hepatotoxicity (Matrix Cell B3). Finally, animal models will allow

the study of how nutritional factors affect liver cytotoxic, injury-response and fibrotic pathways (Matrix Cell A1).

Clinical research: The ultimate goal of research in this area is to translate findings in experimental liver injury, inflammation, repair and fibrosis into meaningful advances in diagnosing, monitoring and treating human liver disease. Towards this goal, better biomarkers for liver disease progression and response to therapy would be helpful. Analysis of proteomic changes in the liver and patterns of liver-derived serum proteins in specific diseases may allow earlier diagnosis and more specific therapeutic intervention (Matrix Cell B2). Ultimately, such information could obviate the need for liver biopsy, an invasive procedure that is currently a mainstay of liver disease evaluation. Also, because industry is reluctant to fund clinical studies that require invasive clinical endpoints that can impact morbidity and mortality, the development and validation of biomarkers to assess ongoing treatment efficacy would greatly stimulate clinical research in liver disease.

To complement biomarker development, efforts to determine the genetic determinants of disease risk and progression in acute and chronic liver injury, fibrosis, and regeneration are also important (Matrix Cell C2). Identification of genetic determinants would refine clinical trial enrollment and shorten treatment intervals by stratifying patients according to risk, allowing for earlier evidence of efficacy. Genetic risk information would also facilitate the transition from preclinical studies to human trials.

Focused and efficient translation of new findings in liver injury, repair, inflammation and fibrosis will depend upon the coordinated development of clinical trials of these new therapies, such as anti-apoptotic and hepatoprotective therapy for viral hepatitis, nonalcoholic steatohepatitis or drug-induced liver injury (Matrix Cells A2 & B3). More challenging is the development of an antifibrotic therapy, an area where long-term studies with careful analysis of hepatic fibrosis and cirrhosis would be helpful (Matrix Cell C3). Definitive, phase III trials of such therapies should only be undertaken after phase I and II trials demonstrate preliminary safety, tolerability, optimal-dose regimen and suitability of surrogate markers of effect. Of great benefit for clinical studies of liver disease and fibrosis would be the development of noninvasive markers for fibrogenesis and the amount of fibrosis (Matrix Cell A3). Elucidation of the pathways of collagen formation, deposition and resolution would help to identify potential candidate markers that might be identified in serum or urine. Use of a noninvasive biomarker rather than liver biopsy to assess development of fibrosis and its resolution would greatly aid the development of therapeutics directed against the fibrotic process.

Steps to Achieve Research Goals

Multifaceted, complementary strategies are necessary to achieve these research goals. An emphasis on investigator-initiated studies would optimally address many of the pathophysiology-related goals. However, larger-scale efforts will be required to identify potential small molecule therapies for liver disease, supported independently of a hypothesis-driven funding format. Such efforts will require mechanisms to encourage industry to partner with academia and invest in liver disease research, while overcoming issues of intellectual property and other legal barriers. The model provided by the National Cancer Institute provides one potential template to surmount these problems. The development of animal models, although resource-intensive, is nonetheless critical to make further advances in the field. A well-funded consortium dedicated to developing relevant and robust animal models of human liver diseases is necessary to achieve this goal. Finally, human studies will require the integration of clinical research networks with investigators versed in proteomics, genomics and molecular medicine. Major emphasis could be given to funding grant applications that focus on the rapid translation of new research findings into innovative but practical approaches to therapy of liver disease. All these vital goals are achievable with a clear and sustained focus and adequate resources.

Matrix of Research Goals in Liver Injury, Repair, Inflammation & Fibrosis

	Short-term Goals (0-3 years)	Intermediate-term Goals (4-6 years)	Long-term Goals (7-10 years)
High Risk	A3. Develop noninvasive biomarkers for fibrosis.	B3. Develop gene, cell-based and pharmacologically targeted therapies for hepatotoxicity.	C3. Develop mechanism-based drug therapy in fibrotic disease, targeting pro-fibrogenic and fibrosis resolution pathways.
Intermediate Risk	A2. Define the role of anti-apoptotic therapy in liver injury, fibrosis and regeneration. Identify the impact of individual leukocyte sub-populations and their mediators on liver injury, fibrosis and regeneration.	B2. Identify the integrative mechanisms mediating oxidative, nitrosative, hypoxic and ischemic-reperfusion injury and the role of sinusoidal cells. Identify the proteomic response of the liver and liver-derived serum proteins as intermediate biomarkers for liver disease progression and response to therapy.	C2. Using high-throughput screens, identify candidate small molecules that modify cytotoxic and fibrotic pathways in liver cells. Define genetic determinants of disease risk and progression in acute/chronic liver injury, fibrosis and regeneration.
Low Risk	A1. Identify individual liver cell type-specific responses to inflammatory mediators. Elucidate whether and how nutritional factors affect liver cytotoxic and fibrotic pathways.	B1. Identify individual liver cell type specific extrinsic (e.g., mediator-based) and intrinsic (e.g., organelle-based) cytotoxic signaling pathways.	C1. Develop relevant and robust animal models of hepatic injury, inflammation, and fibrosis progression and resolution.